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(54) Enzymatic method for the minimization of the content of phosphorous-containing components in vegetable and animal oils

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Description

[0001] The invention relates to a process for the reduction of the content of phosphorus-containing components in edible oils by enzymatic decomposition and separation of the decomposition products. The term edible oil includes vegetable and animal, preferably predemucilaginated, oils.

State of the Art

[0002] The raw soy oil and other raw vegetable oils are subjected to a premucilagination in which phosphatides such as lecithin and other hydrophilic auxiliary components are removed. If this is done by extraction with water, it is also called "wet-demucilagination." In this treatment a portion of the phosphatides remain in the oil, said phosphatides being subsumed under the term "non-hydratable phosphatides" (NHP). For the production of edible oils it is imperative to remove this portion. According to prevailing opinion the phosphorus content should not exceed 5 ppm (cf. Hermann Pardun, "Die Pflanzenlecithine [= Vegetable Lecithins]" Verlag für chemische Industrie, H. Ziolkowsky KG, Augsburg, 1988, Pages 181-194).

[0003] The NHP arise through the action of the plants' own enzymes. These are deactivated in the "Alcon process" by steam-treatment of the soy flakes so that the formation of the NHP is inhibited and in the wet-demucilagination of the raw oil the phosphatide portion can be removed almost entirely.

[0004] From the predemucilaginated oil a significant portion of the NHP can be extracted by means of aqueous surfactant solutions. However, as a rule, one cannot get below 30 ppm. Treatment with acids or alkalies is more successful but requires many operational steps.

[0005] It is a known practice to treat vegetable and animal oils with enzymes whereby enzymatically cleavable components are intended to be decomposed to form water-soluble, easily extracted substances. Thus, according to DE-A 16 17 001, fats for soap production are deodorized with proteolytic enzymes. For the clarification of vegetable oils according to GB 1 440 462, amylolytic and pectolytic enzymes are used. According to EP-A 70 269, animal or vegetable fats or oils in the raw, partly processed, or refined state are treated with one or more enzymes in order to cleave and separate all the components other than glycerides. As suitable enzymes, phosphatases, pectinases, cellulases, amylases, and proteases have been mentioned. As an example of a phosphatase, phospholipase C has been named. The use of enzymes for total delecithinization or total demucilagination, as the removal of NHP from predemucilaginated oils is also called, is not known.

[0006] The nature of the NHP is not precisely known. According to Pardun (loc. cit.) they are lysophosphatides and phosphatidic acids or calcium and magnesium salts formed therefrom which arise through the decomposition of phosphatides under the action of the plants' own phospholipases.

[0007] From JP-A 2-153997 it is a known practice to treat raw or predemucilaginated oil with an enzyme which has phospholipase-A activity. Regulation of the pH value is not provided.

[0008] With regard to the phosphorus content of the starting material as well as the phosphorus content of the end product, no specifications are found in the description. According to Example 1, a demucilaginated oil is treated with an enzyme solution with 3U phospholipase-A activity at a temperature of 60° C for 48 hours. In so doing, the phosphorus content reduced to 1300 ppm phospholipids. Only by treatment at 105° C and 30 Torr with the addition of clay can the phosphorus content be reduced to 40 ppm. According to Example 3, a demucilaginated oil with a phosphorus content of 1638 ppm phospholipid is treated for 30 minutes, 1 hour, 2 hours, or 4 hours at 50° C with a high enzyme concentration and with the addition of 100%, 500%, 1000%, or 2000% water relative to the

amount of oil. In so doing, the phosphorus content after 4 hours is reduced, according to conditions, to 226 ppm, 81 ppm, 153 ppm phospholipid, 206 ppm phospholipids, 179 ppm phospholipid.

[0009] The process for the treatment of oil known from JP-A 2-153997 has the disadvantage that is requires a very long reaction time (48 hours) and the phosphorus content is not sufficiently reduced. The variants in which a further reduction of the phosphorus content is possible, in which clay is added to the reaction mixture, has in addition the disadvantage that the process must be carried out in two steps, that the second step runs at relatively high temperature, and that a vacuum must be applied. Furthermore, an additional filtration step is required. The heating of the oil to a temperature over 80° C is disadvantageous. Oxidation reactions run in the oil and discolor it, which is extremely disadvantageous for its use as an edible oil. Furthermore, from the standpoint of nutrition physiology, the value of oils with a high content in multiply unsaturated fatty acids is partially lost on heating.

[0010] The process described in Example 3 has the disadvantage that large amounts of enzyme and water are required, which must be separated from the oil after the termination of the process. Such conditions are technically and economically impractical since enormous amounts of waste arise and there is the danger that stable oil-in-water emulsions will form. Furthermore, with one exception, the phosphorus content is not sufficiently reduced, even with very high phospholiphase-A activity, the exception being one example in which 500% water relative to the oil was used.

[0011] In JP-A 2-49593 a similar enzyme treatment of oils is described which however is directed not toward the demucilagination of the oil but rather obtaining lysolecithin. Therein the setting of particular pH values is superfluous. Even in the case of processes according to EP-A 328 789 the object is the conversion of the lecithin of the soy to lysolecithin by phospholipase A in the production of mayonnaise-type products.

Objective and Realization

[0012] The aim of the invention is the further reduction of the content of phosphorus-containing and iron-containing components in predemucilaginated animal or vegetable oils to a phosphorus content under 5 ppm by enzymatic decomposition.

[0013] It has been found that the treatment of animal or vegetable oils which have a phosphorus content of 50 to 250 ppm with a phospholipase A_1, A_2, or B in a continuous process leads to phosphorus contents under 5 ppm if the enzyme is treated in the form of an aqueous solution at a pH value of 4 to 6 emulsified to form fine droplets. Phosphorus content values under 5 ppm and iron content under 1 ppm have been achieved. The low iron content is advantageous for the stability of the oil.

[0014] The drastic reduction of the phosphorus content is surprising in so far as the phospholipases A_1, A_2, or B have a pH optimum in the neutral to weak alkaline range. With phospholipase C or D the aim of the process cannot be achieved.

[0015] The object of the invention is thus a process for the reduction of the content in phosphorus-containing components in edible oils by means of a phospholipase. Therein a predemucilaginated animal or vegetable oil with a phosphorus content of 50 to 250 ppm is agitated with an organic carboxylic acid and the pH value of the mixture formed is set to 4 to 6. An enzyme solution which contains phospholipase A_1, A_2, or B is added to the mixture in a mixing vessel under turbulent stirring and with the formation of fine droplets, where an emulsion with 0.5 to 5 % by weight relative to the oil is formed which is conducted through at least one subsequent reaction vessel under turbulent motion during a reaction time of 0.1 to 10 hours at temperatures in the range of 20 to 80° C and where the treated oil, after separation of the aqueous solution, has a phosphorus content under 5 ppm.

Embodiment of the Invention

[0016] Since the phospholipases A_1, A_2, or B would attack lecithin, it is not practical to use oils with high lecithin content such as raw soy oil in the process according to the invention. Starting materials are thus preferably predemucilaginated oils, in particular edible oils which are distinguished as a rule by a phosphorus content between 50 and 250 ppm. Oils of varying quality can be processed on the same equipment. Preferably predemucilaginated oils, above all sunflower oil, rape oil, and in particular soy oil, are used. Previous drying of the oil is imperative.

[0017] The phospholipase is expediently used in aqueous solution which is emulsified as fine as possible. The enzymatic reaction may take place on the boundary layer between the oil phase and the aqueous phase. It is promoted by intensive mixing, for example, by turbulent stirring and in addition by the addition of surfactants. The decomposition products of the NHP have a higher hydrophily and thus go over into the aqueous phase. They are thus removed simultaneously with the aqueous phase just like metal ions.

[0018] Phospholipases A_1, A_2, or B are known enzymes (cf. Pardun, loc. Cit. Pages 135-141). Phospholipase A_1 cleaves the fatty acid ester group at the G_1 atom of a phospholipid molecule. It is found, for example, in the liver of rats and in the pancreas of pigs. From mold cultures of Rhizopus arrhizus, an enzyme with phospholipase-A_1 activity could be isolated.

[0019] Phospholipase A_2, which was previously designated as lecithinase A, cleaves the fatty acid ester group at the C_2 atom of a phospholipid molecule. It occurs, usually in association with other phospholipases, in almost all animal and plant cells. It is found in abundance in the venom of the rattlesnake and the cobra as well as in the venom of bees and scorpions. Technically it can be obtained from pancreas glands, after activity-inhibiting accompanying proteins have been decomposed with trypsin.

[0020] Phospholipase B is widely distributed in nature. It acts on the lysolecithin arising through the phospholipases A_1 action by cleaving the second fatty acid ester functional group. In part it is also considered as a mixture of the phospholipases A_1 and A_2. It occurs in the liver of rats and is also produced by many molds such as Pencillium notatum.

[0021] Phospholipases A_2, or B are available as commercial products. For technical application the use of the purified enzyme is not required as a rule. For the process of the invention a phospholipase preparation is suitable which is obtained from ground pancreas gland pulp and above all contains phospholipase A_2. The enzyme is—according to activity—used in amounts of 0.001 to 1 % by weight relative to the oil. A good distribution of the enzyme in the oil is assured if it is dissolved in an amount of water of 0.5 to 5 % by weight relative to the oil and is emulsified in this form in the oil to form droplets of less than 10 µm in diameter (average value of weight). Turbulent stirring with radial speeds over 100 cm/s has proven itself effective. Instead of this, the oil can be circulated in the reactor with the aid of an external turbine pump. The enzymatic reaction can also be promoted by means of the action of ultrasound.

[0022] The enzyme action is increased by the addition of an acid, preferably an organic carboxylic acid, which is added before the enzyme treatment. Citric acid is preferred. It can be used in the form of the free acid or as a buffer system in combination with its salt, such as an alkali metal salt (for example, sodium citrate), an alkaline earth metal salt (for example, calcium citrate), or an ammonium salt. Suitable amounts are 0.01% by weight relative to the oil, optimally 0.1% by weight. With the acid the pH value is set to a value of 3 to 7, preferably from 4 to 6. The optimum lies at about pH 5. Surprisingly this pH value is also optimal when the phospholipase is used in the form of an enzyme complex from the pancreas. The pancreas enzyme complex otherwise has a pH optimum of 8 and is hardly active at all at pH 5. Apparently a higher pH value is set at the phase boundary layer, where the enzyme action occurs, than within the aqueous phase.

[0023] In order to bring the phospholipases A_1, A_2, or B from the fat-containing pancreatin or pancreas products into solution, emulsifying additives are helpful. Water-soluble emulsifiers are suitable, in particular those with an HLB value over 9 such as Na-dodecylsulfate. It is active in an amount of, for example, 0.001% by weight relative to the oil if it is added to the enzyme solution before the emulsification in the oil.

[0024] The addition of additional enzymes, above all proteinases and amylases, often has an advantageous effect. Also protein additives can be advantageous due to a certain surfactant action.

[0025] The temperature in the enzyme treatment is not critical. Temperatures between 20 and 80° C are suitable. A temperature of 50° C is optimal but short-term heating up to 70° C is possible. The period of treatment depends on the temperature and can be kept shorter with increasing temperature. Times of 0.1 to 10, preferably 1 to 5 hours are sufficient as a rule. A step program has proven itself particularly effective, in which the first step is executed at a temperature of 40 to 60° C and the second step at a higher temperature in the range of 50 to 80° C. For example, agitation is done first for 3 hours at 50° C and then for 1 hour at 75° C.

[0026] After the conclusion of the treatment, the enzyme solution with all the decomposition products of the NHP incorporated therein is separated from the oil phase, preferably by centrifuging. Since the enzymes distinguish themselves by high stability and the amount of decomposition products taken up is small, the same enzyme solution can be reused repeatedly.

[0027] Preferably the process is carried out continuously. In an expedient, continuous mode of operation the oil is emulsified with the enzyme solution in a first mixing vessel, allowed to react, under turbulent motion, in one or more subsequent reaction vessels, in some case at increasing temperature, and the aqueous enzyme solution subsequently separated in a centrifuge. In order to avoid an enrichment of the decomposition products in the enzyme solution, a portion thereof can be replaced by fresh enzyme solution on an on-going basis and the rest recycled into the process.

[0028] The oil obtained has a phosphorus content under 5 ppm and is thus suitable for physical refining. Thanks to the low iron content achieved, it has good prerequisites for achieving high resistance to oxidation during refining.

EXAMPLES

Example 1

[0029] 1 l of wet-demucilaginated soy oil with a residual phosphorus content of 110 ppm is heated to 75° C in a round flask. Under strong stirring with a blade stirrer 5 cm in diameter at 700 rpm, 10 ml of water containing 1 g of citric acid are added and stirred further for 1 hour. Then there is cooling to 40° C and a solution of 0.1 g phospholipase A_2 of the quality specified in [sic] Example 1 as well as 50 mg of calcium chloride in 20 ml of 0.1 molar acetate buffer pH 5.5 are added. After a further 5 hours of intensive stirring the aqueous phase is centrifuged off. The contained oil contains 2 ppm and is suitable for physical refining. The change of the other characteristic numbers follows from the following table:

	Starting Material	Treated Oil
Phosphorus	110 ppm	2 ppm
Iron	3.30 ppm	<0.1 ppm
Calcium	65.4 ppm	5.3 ppm
Magnesium	38.4 ppm	<0.1 ppm
Peroxide number	18.3	18.50
Acid number	0.91	1.10
Saponification number	191.2	190.4

Example 2

[0030] The process according to Example 1 is repeated with the difference that instead of phospholipase A_2 1 g of a pancreas preparation (pancreatin, 800 phospholipase units/g) is used. The preparation contains phospholipase A_2, proteinase, amylase, lipase. The phosphorus content sinks to under 1 ppm. Due to the influence of the lipase only a negligible increase of the acid number from 0.91 to 1.49 occurs.

Example 3

[0031] 9 l of wet-demucilaginated rape oil with a phosphorus content of 72 ppm is mixed with a solution of 8.6 g of citric acid in 250 ml of water and heated to 60° C. The mixture is homogenized by being circulated by means of an external turbine pump once per minute. Then the pH value of the aqueous phase is set to 5.0 with 30 g of 10% sodium hydroxide solution. Then 9 g of phospholipase A_2 with an activity of 400 U/g together with some calcium chloride are added and the mixture circulated at 60° C for 3 hours in the specified manner. After centrifuging off, a phosphorus content of 3 ppm is found.

Claims

1. Process for the reduction of the content process for the reduction of the content of phosphorus-containing components in animal and vegetable oils by enzymatic decomposition by means of a phospholipase, therein a predemucilaginated animal and vegetable oil with a phosphorus content of 50 to 250 ppm is agitated with an organic carboxylic acid and the pH value of the resulting mixture set to 4 to 6, an enzyme solution which contains phospholipase A_1, A_2, or B is added to the mixture in a mixing vessel under turbulent stirring and with the formation of fine droplets, where an emulsion with 0.5 to 5 % by weight relative to the oil is formed, said emulsion being conducted

through at least one subsequent reaction vessel under turbulent motion during a reaction time of 0.1 to 10 hours at temperatures in the range of 20 to 80° C and where the treated oil, after separation of the aqueous solution, has a phosphorus content under 5 ppm.

2. Process according to claim 1 characterized by the fact that a wet-demucilaginated oil is used.

3. Process according to claim 1 or 2 characterized by the fact that, as organic carboxylic acid, citric acid in the form of the free acid or as alkali metal, calcium, or ammonium salt is used.

4. Process according to one or more of the claims 1 to 3 characterized by the fact that in addition an emulsifier is used.